

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2015 – 2017**

TITLE: Identifying Lethal Temperatures Targeting Immature Life Stage Control of Spotted Wing
Drosophila

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SUMMARY: Survival of spotted wing drosophila (*D. suzukii*, SWD) larvae to adulthood was evaluated after exposure to temperatures ranging 29-49°C in a laboratory thermal gradient bench for 60 minutes. Vials filled with pre-made fly diet up to approximately 1.5 cm from the bottom were used as oviposition media inside the gradient wells. Forty-three vials were prepared each day for 4 consecutive days. Five adult females were placed in each of 36 vials for 24 hours for oviposition to occur, seven vials were held as controls without flies. Upon removal, adult flies were placed in ethanol. Four ages were tested between 1-4-day old egg/larvae (i.e. 1 day old, 2 days old, 3 days old, and four days old). Sample vials were examined for oviposition success. Immature flies exposed temperatures of 32-35°C had the highest survival to adulthood. Above 38°C survival to adulthood with no survival at 41°C and 49°C, and a few surviving to adulthood under 45°C. An additional trial was conducted to evaluate the potential for thermal conditioning to improve larval survival at higher temperatures. For this study, four-day-old larvae were subject to 30, 60, and 90 minutes at 35°C, then allowed to cool to room temperature (22°C) for 60 minutes prior to placement in the thermal bench. These trials were used to determine how heat may reduce successful development to adulthood, leading to better management practices in the field.

OBJECTIVES: The objective of this study was to determine the temperature at which SWD larval development declined and mortality increased leading to better management practices for population control. Currently, control of SWD in commercial crops depends on abundant use of insecticides. Canopy manipulation or installing black weed mat to increase temperature could potentially reduce larval and pupal development by increasing temperatures.

PROCEDURES:

Source material

Approximately, 186 Fisherbrand disposable culture tubes (size 16 x 100 mm, Stock no, Fisher Scientific, Pittsburgh, PA) were used in both experiments. Yeast-based fly diet was prepared and poured into the bottom of each vial filling to approximately 1.5 cm (Dalton et al. 2011). For each trial, 2-inch insulation board (Item # 45W, Owens Corning, Ohio) was cut to size and used as stoppers to prevent larval escape from the enclosed tubes containing the fly diet. The

insulation corks were used in the initial trial. Parafilm was utilized following thermal bench exposure for the second trial with ventilation holes.

Adult emergence after larval heat exposure

D. sukii eggs and larvae were obtained from mated females which came from stock colonies that were continuously supplemented with field-collected individuals in commercial fields starting November 2009. The trial was designed to allow for four egg-larval age groups in order to see if age played a role in larval survival rates or response to pre-treatment heat therapy. For each age group of immature flies, a total of thirty-two individual vials received eggs from the ovipositing females and eleven vials served as controls (44 vials per day). In all, there were 132 vials. The flies placed into the tubes were allowed to oviposit for 24 hours before they were removed. The vials were enclosed to prevent adult escape. The heat treatment experiments started on day 5 after the experiment was initiated with the ovipositing females.

Larvae exposed to the respective heat treatments ranged in age from 1-4 days old. All vials were kept in laboratory at 22°C until they were moved to the thermal gradient bench. Eight vials from each day, 4 infested and 4 control, were kept in laboratory conditions at 22°C for the duration of the experiment as a standard control. On the fifth day, the remaining 28 vials of larvae and the 7 control vials were placed in the thermal gradient bench (Bubliy et al 2013), which maintained constant temperatures ranging from 29°-49°C (Figure 1). Seven row positions of a custom-made solid aluminum heat bench (A-G), each with its unique temperature contained 20 wells (give diameter etc) per row. Vials containing the 1-4 day old larvae were placed within to expose larvae for a period of 60 minutes (Bubliy et al. 2013) (Figure 1). Temperatures within vials containing the media were measured using a thermocouple sensor that was inserted into the media and measured every minute using a thermocouple temperature logger (Model # UX120-014M, Onset Computer Corporation, Bourne, MA). Temperature probes were placed within 2 vials in each row. The mean temperature for each row was averaged for the experimental period (Figure 1). The thermal gradient bench exposed the vials to temperatures surrounding the vials so the contents of the vials were enclosed on all vertical and the ventral side of the vial for each of the 7 temperatures (Boher et al. 2012).

Adult emergence after larval heat therapy and exposure

All methods from the first trial were followed except only 4-day-old larvae that were heat conditioned at 36°C in the heat bench for 0, 30, 60, or 90 minutes. After the heat conditioning, the vials containing the larvae were returned to the laboratory (22°C) for 60 minutes. After 60 minutes of cool-down, the vials were placed along the 28-49°C temperature gradient for 60 minutes.

Vials containing larvae in both experiments were then transferred to 22°C where *D. sukii* were reared for 11-21 days. Emerging adults were individually counted and removed on a daily basis during this period. All stages and controls were randomized in their position for each row. Each row had 4 vials from each of the 4 ages including the 4 controls filling all 20 slots per row.

Statistical analysis

Effect of temperature on the number of adult emergence individuals that emerged after heat exposure was analyzed using ANOVA with temperature and heat therapy treatments as independent factors. Differences of the means for significant ANOVA analyses were separated using Tukey's HSD.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

Adult emergence after larval heat exposure

The overall survival of adult *D. sukukii* emerging from all the treatments showed a clear trend (Table 1.). The mean number of adults emerging from 29-38°C ranged from 0.02-12.06 individuals per vial. *D. sukukii* emergence levels at 29-38°C were statistically similar.

Emergence levels were however significantly higher than the number of adults emerging at temperatures above 38°C (F6, 313=56.74, p<0.001). The only temperature above 38°C where *D. sukukii* emerged, was 45°C. At this temperature, most vials had zero emergence, however, 2 of the 48 vials had 3 adult *D. sukukii* emerge. Although statistically not significant, this is of biological importance.

Adult emergence after larval heat therapy

Treatment effects. Of the four treatments, mean adult emergence for no heat conditioning ($\bar{x} = 2.94 \pm 0.73$), and 90 minutes ($\bar{x} = 2.74 \pm 0.7$), were statistically similar for those two treatments (F1, 68=0.39, p=0.84). The 30 minute (Mean adult emergence = 5.46 ± 0.92) and 60 minute (Mean adult emergence = 4.69 ± 0.75), resulted in similar higher emergence rates (F1, 68=0.42, p=0.52). For this reason, we decided to treat the statistically similar groups "no therapy benefit" and "high therapy benefit" To say suboptimal vs optimal suggests greater interpretation than you have. Statistical analysis of 'less optimal' and 'more optimal' groups was statistically different (F 1, 138=8.28, p<0.005) with lower numbers of 'less optimal' treated flies emerging (Mean adult emergence = 2.83 ± 0.5) compared to 'more optimal' treated flies (Mean adult emergence = 5.07 ± 0.59). Statistical differences in emergence levels were also found when comparing adult emergence at different temperatures (F 6, 126=3.7, p<0.005, Figure 2.). Here more flies emerged from 'more optimal' treatments at 32 and 38°C. At 41-48°C, the only treatments where adult *D. sukukii* emerged, were those that received 30 and 60 mins conditioning treatments. The 30 and 60-minute conditioning treatments show a trend of higher adult emergences at nearly every temperature.

Effect of insect gender

The gender of insects had a significant effect on adult *D. sukukii* emergence (F 6,126=3.7, p<0.005, Figure 3.). Mean male *D. sukukii* emergence was numerically higher for no or 90 minutes of heat conditioning treatments and statistically higher than those of females for 30 and 60-minute heat conditioning treatments. When comparing the effect of temperature for males, males emerged at higher rates for more optimally treated individuals at 29, 32 and 38°C (Figure 4.). Males also were able to emerge at 41, 45 and 48°C. When comparing the effect of temperature for females, they emerged at higher rates for 30 and 60-minute heat conditioning

treated individuals at 32 and 38°C (Figure 5.). Females also were able to emerge at 41 and 45°C, but not 48°C.

Discussion

These findings provide an understanding of *D. suzukii* thermal tolerance. This information can be used to more optimally manage larval *D. suzukii* under field conditions. Late instar larval *D. suzukii* are known to drop from fruit onto the ground before pupation (Meghan Woltz, personal communication). Fruit growers use cultural practices including the use of weed fabric that can act as the interface between larvae and pupae and can be used to manage *D. suzukii*. This study was conducted in order to improve understanding of survival of *D. suzukii* larvae exposed to extreme temperatures as found on black weed fabric which are known to reach temperature above those tested in our experiments (V. Walton pers. Obs.). We found that survival of extreme temperatures can be improved after heat conditioning treatments.

In initial trials, *D. suzukii* received no heat conditioning and there was a biological threshold of larval survival at around 38°C. At temperatures above this level, larval mortality increased significantly. A possible explanation for the numerical increase in survival levels at 49°C could be genetic variability (Murphy et al. 2016) as described for many drosophilids. A study by Goto and Kimura (1998) described the benefit of heat-shock proteins (Hsps) that are synthesized in response to extreme heat or cold. The production of Hsps comes at a significant cost to cell growth and fecundity, but allows for survival under extreme conditions. This may explain how the larvae exposed to sublethal conditions were able to survive the exposures in the thermal bench. Goto and Kimura (1998) trialed three *Drosophila* species and results showed that the highest accumulations of Hsps were recorded between 29°-36°C with strong declines outside of these temperatures for flies that did not receive exposure to extreme environmental conditions similar to heat conditioning treatments used in our study. Feder et al. (1996) found that by exposing *D. melanogaster* larvae to 36°C, prior to higher temperature exposures, extreme thermal survival was significantly improved. Their study replicated conditions the flies would experience in the wild lending valuable knowledge on how Hsps plays an active role in survival for these species. Dillon et al. (2009) suggest that *Drosophila* species, and other flies, choose when to oviposit for the best developmental time of their brood (see previous chapter). *Drosophila* species may prefer afternoon temperatures at or just after the hottest point in the day, allowing for eggs to hatch and begin larval development within 24 hours after oviposition, thereby increasing chances for survival.

The literature indicates that exposure to upper and lower thermal thresholds conditions may provide a benefit to immature insect survival rates under extreme field environments. Our data suggest that *D. suzukii* larvae are more tolerant to thermal extremes when exposed to heat conditioning for short periods before being exposed to extreme heat. However, there was no benefit when *D. suzukii* was exposed to periods of 90 minutes. Our data also shows a difference in benefit between sexes, with male *D. suzukii* emerging at higher rates compared to females after heat conditioning and extreme temperature exposure. Condon et al. (2015) found thermal tolerance to be sexually dimorphic in a trial with *D. melanogaster*. They found males resisted exposure to heat longer and females recovered from chill coma quicker. They also concluded from their study that wing size and thermal tolerance couldn't be used to determine a relationship between sexual dimorphism of thermal tolerances. Although they did not find

fecundity variation in regards to heat tolerance, there was a marginally significant interaction between cold tolerance and selective population. They also concluded that flies reared at 25°C were better able to adapt to heat and cold extremes versus the flies that were reared at temperatures above or below this level, or by varying temperatures. A study conducted on two populations of *Drosophila buzzatii* from Argentina, one from a lowland associated with high temperatures and one from a highland with lower temperatures of, concluded that the flies had numerous traits that were different and were explainable by temperature differences. Egg laying times were different and were aligned with the differing daytime temperatures in both locations. After exposing both populations to sublethal temperatures, it was found that when flies were exposed to 39°C, the highland population expressed an increased Hsp70 (Sorensen et al. 2001). This study suggests temperature adaptation of a population where one may be better fit to survive temperature extremes than another from the same species. This could explain differing intraspecies oviposition behavior between regions.

Activation of Hsp70 gene expression has been associated with improved thermotolerance in other organisms beyond *Drosophila* species and insects. In a trial conducted on chicken eggs and the chicks thereafter hatching, it was found that the chicks that were exposed to higher temperatures during embryogenesis expressed Hsp70 and were associated with having improved thermotolerance when exposed to higher temperatures as chicks, as well as, significantly higher body weight than those that were kept at ideal temperatures (Al-Zhgoul et al. 2013). In a study involving rats, it was tested if Hsp70 expression would differ between exercise induced or sedentary with and without increased temperatures. The rats that were exercising and sedentary in higher temperatures had higher levels of Hsp70 expression. They concluded that exercise alone was not sufficient to activate Hsp70 expression suggesting that Hsp70 may be activated by increased ambient temperature, not just internal temperature from motion (Walters et al. 1998). Even small life forms such as protistan parasites, *Blastocystis* sp., were studied for Hsp70 expression under temperature extremes. It was found that the cultures that were exposed to thermal extremes of 41°C, then returned to 37°C had doubled compared to controls and had increased expression of Hsp70 (Gaythri et al. 2014).

This work highlights some mechanisms used by *D. suzukii* to survive suboptimal environmental conditions. A trial in which the temperature in heat conditioning is slowly increased would be beneficial to determine the temperature threshold where mortality increases and developmental success declines. Future work should also evaluate SWD for Hsps mRNA within their genome to determine possible success in regulating proteins for increased temperatures. *D. suzukii* DNA holds information on their ability to adapt to varying climatic conditions. In future trials, having a known number and age of larvae and pupae prior to thermotolerance testing would result in a true success rate in relation to temperature exposure. The flies that survived to adulthood could be tested for their Hsps levels to determine the success of *D. suzukii* in a changing climate and potential pitfalls to new management strategies involving increasing temperatures. Transferring *D. suzukii* from a 22°C controlled environment to extreme heat temperatures for a brief time period doesn't occur naturally and therefore does not accurately reflect what happens under natural conditions. These data do however provide baseline knowledge that provides an indication of the thermal thresholds of *D. suzukii*.

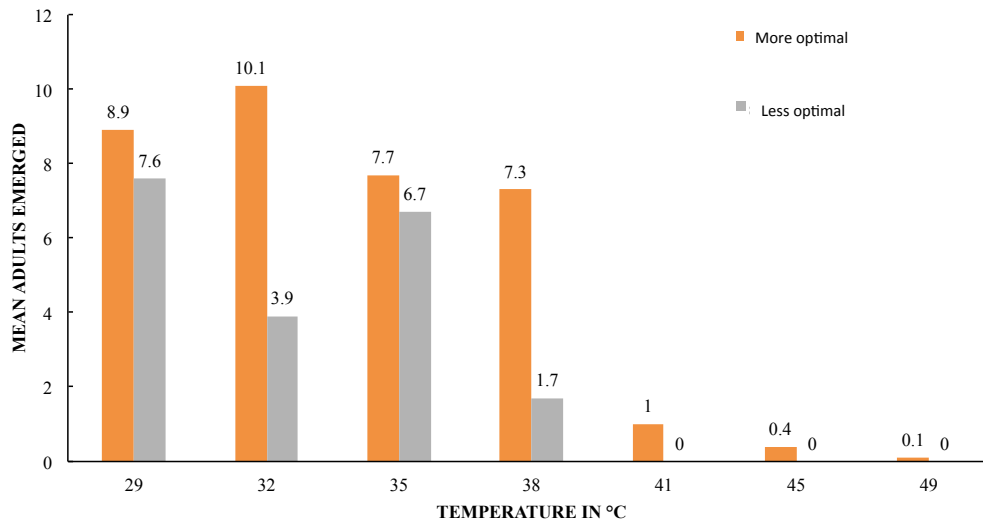


Figure 2. Mean adult *D. sukuzii* emergence over 30-minute temperature exposure. Individuals were exposed to no and ninety (less optimal), and thirty and sixty (more optimal) heat therapy treatments at 36°C. All individuals were allowed a sixty-minute recovery period at 22°C. after which they were placed for 60 minutes at 29-49°C.

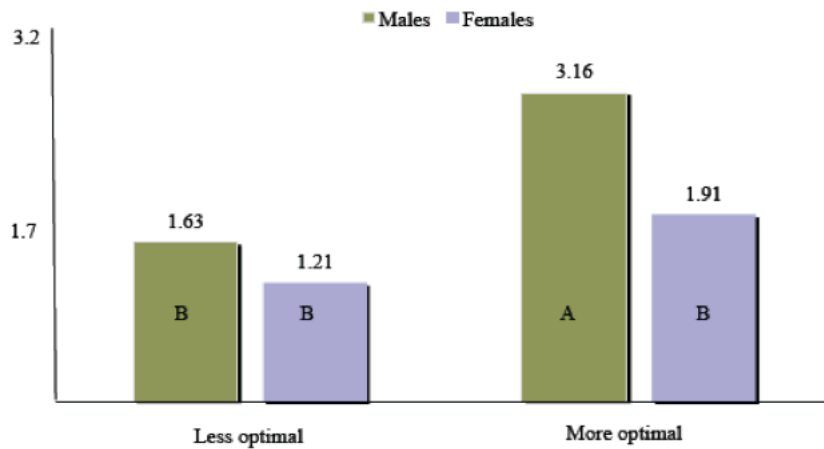


Figure 3. Comparison of mean adult male and female *D. sukuzii* emergence after larval treatments for more optimal and less optimal heat therapy treatments. Bars with significant differences have different letters (F6,126=3.7, p=0.002).

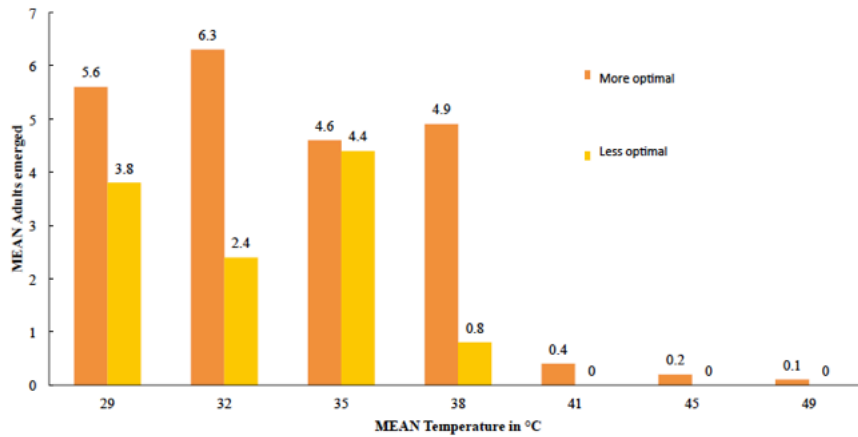


Figure 4. Mean adult male *D. sukukii* emergence after no and heat conditioning treatments. Individuals were exposed to no and ninety (less optimal), and thirty and sixty (more optimal) heat conditioning treatments at 36°C. All individuals were allowed a sixty-minute recovery period at 22°C. after which they were placed for 60 minutes at 29-49°C.

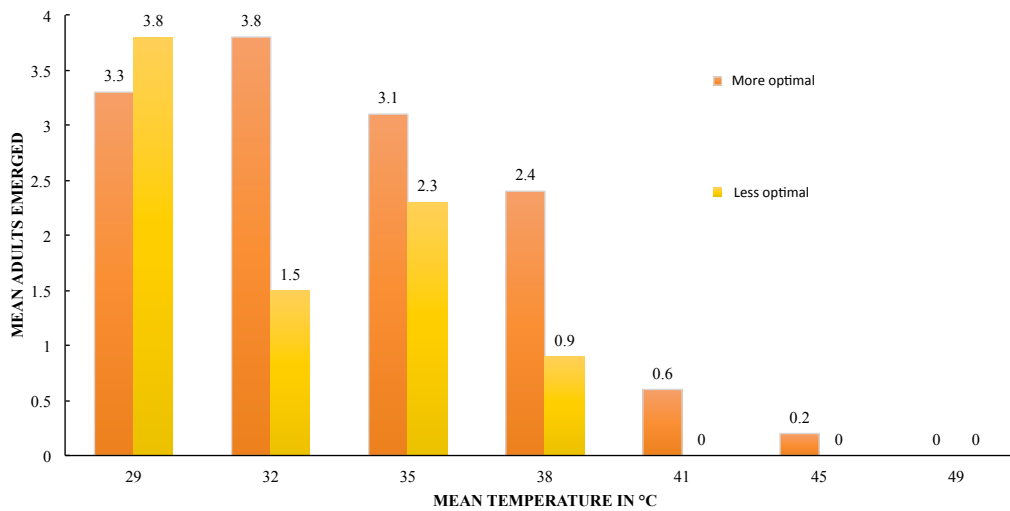


Figure 5. Mean adult female *D. sukukii* emergence after no and heat conditioning treatments. Individuals were exposed to no and ninety (less optimal), and thirty and sixty (more optimal) heat therapy treatments at 36°C. All individuals were allowed a sixty-minute recovery period at 22°C. after which they were placed for 60 minutes at 29-49°C.

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